COMPARATIVE EVALUATION OF DIFFERENT CYCLODEXTRINS AND METHODS OF PREPARATIONS FOR IMPROVING ORAL BIOAVAILABILITY OF BCS CLASS II DRUGS-SAQUINAVIR AND RITONAVIR

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The embodied work in this Ph.D. thesis describes comparative studies on the complexation of saquinavir and ritonavir with different cyclodextrin derivatives using different methods of preparation for the enhancement of solubility, dissolution rate and oral bioavailability.

Human immunodeficiency virus (HIV) infection, which leads to acquired immunodeficiency syndrome (AIDS), remains a serious worldwide health problem. The Government of India estimates that about 2.40 million Indians are living with HIV (1.93 -3.04 million) with an adult prevalence of 0.31%. Children (<15 yrs) account for 3.5% of all infections, while 83% are the in age group 15-49 years. India's highly heterogeneous epidemic is largely concentrated in only a few states in the industrialized south and west, and in the north-east. The discovery of HIV protease inhibitors introduced new and effective first line therapies for HIV/AIDS. Helping to combat HIV-related diseases and prolong survival, protease inhibitors are commonly administered with reverse transcriptase inhibitors. However, poor patient compliance, noxious side effects, and viral resistance have led to a recommendation to treat with different kinds of protease inhibitors.

The most important HIV protease inhibitors in clinical use are saquinavir, nelfinavir, indinavir, lopinavir, ritonavir, atazanavir, and amprenavir. These protease inhibitors are metabolized by cytochrome P450 3A (CYP3A) enzymes, are efflux transporter substrates (i.e., P-glycoprotein, P-gp), or both. These metabolism and transport mechanisms often result in widely variable drug absorption. In addition to the metabolism and transport issues, many protease inhibitors have poor aqueous solubility,

which produces very low and variable bioavailability. As a result, HIV/AIDS patients require frequent and large medication dosing and commonly are unable to adhere to their treatment regimes.

An archetypal protease inhibitor, saquinavir has poor water solubility and is reported to be an excellent P-gp and CYP3A substrate. As a result, the oral bioavailability has been reported to be very low (0.7-4.0%) and dependent upon the dosage form used. Ritonavir, a widely prescribed antiretroviral protease inhibitor drug belongs to class II under BCS and exhibits low and variable oral bioavailability due to its poor aqueous solubility. Its oral absorption is dissolution rate limited and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. Ritonavir, a CYP3A and P-gp inhibitor, helps to increase saquinavir oral bioavailability. However, because HIV/ AIDS patients must take other drugs known to be metabolized by CYP3A or they are P-gp substrates, ritonavir has been shown to cause additional toxicity and safety issues. Therefore, novel pharmaceutical formulations that may safely enhance the bioavailability of protease inhibitors are needed.

Several approaches could be investigated to improve their oral bioavailability, among them, complexation with cyclodextrins is drawing considerable attention these times, because of their low toxicity, low cost, biocompatibility, biodegradability and abundant availability. Cyclodextrins are cyclic (R-1, 4)-linked oligosaccharides of R-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. CDs and their derivatives have received considerable attention in the

pharmaceutical field for the past few years and an increased number of reviews have been dedicated to their industrial and pharmaceutical applications.

Drug-CD complexation and improvement in solubility and dissolution is influenced by both nature of the cyclodextrin (native or chemically modified, crystalline or amorphous) and the method of complexation, viz co-grinding, kneading, solid dispersion, solvent evaporation, co-precipitation, spray drying or freeze drying. The effectiveness of a method depends on nature of the drug and CD. In many cases, spray drying and freeze drying were found to be more effective for drug complexation.

Therefore, the interest of the present investigation is to prepare series of binary systems of saquinavir and ritonavir with crystalline native β cyclodextrin and its amorphous and highly soluble derivatives, HPβCD, RMβCD and SBE7βCD for improving their solubility. The main aim of the study is to find out the effectiveness of the method of preparation and better cyclodextrin derivative in enhancing solubility and dissolution of saquinavir and ritonavir so that their bioavailability can be enhanced giving scope for reduction in drug dosing for minimizing side effects.

The major objectives of the present investigation are a) to conduct phase solubility studies of selected drugs with different cyclodextrins for the calculation of stability constants of drug-CD complexes and molar ratio of complex formation. b) To prepare saquinavir and ritonavir cyclodextrin complexes using methods like physical mixing, kneading, solvent

evaporation, physical mixtures, spray drying and freeze drying. c) To evaluate prepared CD complexes for their drug content and reproducibility of the method. d) To conduct *in vitro* dissolution studies for the prepared complexes and their optimization for complete drug release in minimum time i.e. not more than 60 min based on the dissolution studies, and on different dissolution and other parameters. e) Identification of best optimized drug-CD complex by using statistical approaches like analysis of variance (ANOVA), Tukey multiple comparison test and their evaluation for stability. f) Characterization optimized complexes for formation of inclusion complexes, drug-CD interaction studies using Infrared spectroscopy (IR), Differential Scanning Calorimetry (DSC), X-Ray Diffraction studies (XRD) and nuclear magnetic resonance spectroscopy (NMR). g) To evaluate the *in vivo* performance of the optimized complexes in comparison with the pure drug using suitable animals like Wistar rats.

The thesis consists of five chapters. Brief introduction about complexation, their preparation and applications in improving oral bioavailability of various drugs is dealt in **Chapter I.**

In **Chapter II**, physicochemical, pharmacological and therapeutic profiles of saquinavir (SQV) and ritonavir (RTV) are described. A brief review of the past work on the improvement of dissolution rate and oral bioavailability of these two drugs is given.

The *in vitro* and *in vivo* analytical methods used in the present investigation for the selected drugs are described. UV spectrophotometric methods reported in the literature were used for *in vitro* estimation. These

were found to be reproducible, robust as per the studies carried out. The reported LC-MS/MS method was validated and used for estimation of the drugs in rat plasma during *in vivo* studies. Profiles of different derivatives of cyclodextrins used were also described.

Chapter III describes the preparation and evaluation of inclusion complexes of saquinavir and ritonavir by using different cyclodextrin derivatives. Phase solubility studies were conducted to calculate the stability constant. The results indicated the improvement in the solubility of these drugs to a greater extent. The phase diagrams obtained were of A_L type indicating formation of 1:1 complex. So, 1:1 ratio was fixed for preparation of all complexes. Different methods of preparation and different CDs were tried in enhancing dissolution rate. The complexes were prepared by using methods like physical mixing (PM), co evaporation (COE), kneading technique (KN), spray drying (SD) and freeze drying (FD). Saquinavir complexes were prepared using βCD, HPβCD, RMβCD and SBE7βCD by PM, KN, COE, SD and FD methods. In case of ritonavir, HPβCD and RMβCD were tried with PM, COE, SD and FD.

Physical mixture (PM) of CD and drug was prepared by simple blending of the materials. Kneaded complex (KN) was prepared upon kneading the moistened materials. Co evaporated complex (COE) was prepared by evaporation of solvent. Spray drying technique (SD) was used by evaporating solvent in a spray drier. Freeze drying (FD) technique was adopted by freezing the drug-CD solution followed by drying. The prepared complexes were passed through sieve no. 80. All the complexes prepared

were found to be fine powders with good flow properties. Low % CV values in the percent drug content ensured drug content uniformity in each batch and reproducibility of the methods of preparation. The drug content obtained was in the range of 65-70%.

The complexes obtained were evaluated for drug release studies by performing the dissolution rate studies. The dissolution rate of drugs is dependent on the method of preparation and type of CD used. Various dissolution parameters like DE_{60}, DP_{30}, DE_{10}, T_{50} and MDT values were calculated and subjected to ANOVA and Tukey multiple comparison test to optimize and to identify the best complexes. The results of ANOVA showed statistically significant difference among different methods with pure drugs. Significant difference was also observed with different cyclodextrins.

Complete drug release was observed in 60 min for saquinavir-SBE7βCD compexe prepared by freeze drying technique. Between HPβCD and RMβCD used for ritonavir complexation, RMβCD complex prepared by freeze drying showed rapid release of drug within 10 minutes and these two were considered as optimized drug-CD complexes and subjected for further studies.

The optimized complexes were subjected to drug interaction studies like FTIR, DSC, ^{1}H NMR, XRD and SEM. The FTIR studies indicated complexation of the drugs with the CDs used in the present investigation in all the methods. The XRD studies indicated the reduced crystallinity of the drugs in presence of CDs indicating the possible conversion of the drugs into amorphous form thereby increase in the dissolution rate of the

drugs. The DSC studies also indicated the possible formation of complex. ^{1}H NMR studies proved inclusion of both the drugs into CD cavity. Optimized complexes and pure drugs were filled into capsules to find out the suitability of converting into a dosage form. The capsules were subjected to uniformity of weight test and dissolution studies. All capsules were uniform in weight and showed similar dissolution profiles compared to the powdered complexes.

Chapter IV describes stability studies which were conducted on the selected complexes, saquinavir complex with SBE7βCD prepared by freeze drying and ritonavir complex with RMβCD as per ICH guidelines. No visible physical changes were observed in all the formulations withdrawn from the stability chambers. The average drug content in all the formulations was found to be satisfactory. The drug release profiles of all the formulations did not change significantly after storage at 25±2°C/60±5%RH and 40±2°C/75±5%RH for a period of six months. The release profiles were found similar for saquinavir after calculating f_1 and f_2. The dissolution profiles of ritonavir remained unaltered.

Chapter V describes the *in vivo* evaluation studies of saquinavir and ritonavir formulations carried out on healthy male Wistar rats. An independent Ethics Committee of Sree Siddaganga College of Pharmacy, Tumkur approved the study. The animals were divided into four groups, each group containing four animals. Two groups were administered with pure drugs and two groups with the complexes. The complex was dispersed in gum acacia solution and administered orally through oral feeding tube.

Serial blood samples (1-1.5 mL) were collected in micro centrifuge tubes containing EDTA coated tubes. Amount of saquinavir and ritonavir present in the plasma samples was estimated by LC-MS/MS method. *In vivo* evaluation test was also conducted for pure saquinavir. Similarly, *in vivo* studies were carried out for prepared ritonavir-RMβCD complex and pure ritonavir. From the bioavailability studies the common pharmacokinetic parameters such as maximum plasma concentration (C_{max}) and time of its occurrence (T_{max}), elimination rate constant (K_{el}), elimination half-life ($t_{½}$), area under the curve (AUC), clearance (Cl/F), volume of distribution (V_d/F) were calculated. The results of *in vivo* tests indicated that the bioavailability of prepared saquinavir complex containing SBE7βCD and ritonavir complex with RMβCD has been increased significantly when compared to the bioavailability of pure drugs. Hence, SBE7βCD for saquinavir and RMβCD for ritonavir could be used as promising cyclodextrin derivatives in the development of solubility and dissolution rate enhancement studies of protease inhibitors, saquinavir and ritonavir. Among various methods used to prepare CD complexes, freeze drying was found ideal with good interaction between CD and the drugs used in the study and also converting the drug into highly amorphous form.

Significant contributions of the present investigation

1. For the first time, comparative studies with different CDs like SBE7βCD and RMβCD for improving bioavailability of BCS class II drugs, saquinavir and ritonavir were carried out.
2. Simultaneously studied the effect of method of preparation and influence of CD on enhancement of solubility and dissolution.

3. Quantities of CDs were minimized in the preparation of drug-CD complexes with the help of phase solubility studies.
4. With the help of FTIR, DSC, ^{1}H-NMR, XRD and SEM studies formation of inclusion complexes was established.
5. Freeze drying method was found to be useful in improving the solubility and dissolution of both the drugs and the *in vivo* studies confirmed the improvement in oral bioavailability of these drugs.

Further studies on these complexes may lead to reduction of the dose of these drugs due to improved bioavailability. A summary of the research work and conclusions drawn are given at the end of the thesis.

In conclusion, this study clearly indicated the applicability of the preparation of inclusion complexes for improving the dissolution rate and oral bioavailability of saquinavir and ritonavir.

The source of information of references cited in thesis is given at the end of each chapter. Overall the entire thesis covers a brief introduction, a detailed description of the experimental methods, results and discussion.